Bacterial cell division: **A moving MinE sweeper boggles the MinD**

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Placement of the division site in *Escherichia coli* is determined in part by three Min proteins. Recent studies have shown that MinE, previously thought to form a static ring near the division site at the midcell position, actually joins MinC and MinD in their rapid oscillation between the cell poles.

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Cytokinesis needs to be regulated spatially in order to ensure that it occurs between the daughter genomes. In prokaryotes such as *Escherichia coli*, cytokinesis is initiated by FtsZ, a tubulin-like protein that assembles into a ring structure at the cell center called the Z ring. A fundamental problem in prokaryotic cell biology is to understand how the midcell division site is identified. Two major negative regulatory systems are known to be involved in preventing Z-ring assembly at all sites except the midcell. One of these systems, called nucleoid occlusion, blocks Z-ring assembly in the area occupied by an unsegregated nucleoid until a critical stage in chromosome replication or segregation is reached. The other system consists of three proteins, MinC, MinD and MinE, which prevent assembly of Z rings in regions of the cell not covered by the nucleoid, such as the cell poles. Recently it was shown that MinC and MinD undergo a remarkable rapid oscillation from one cell pole to the other, but MinE appeared to be fixed near the cell center. More recent results now suggest, however, that all three Min proteins actually oscillate together, with MinE chasing MinC and MinD back and forth across the cell. Possible mechanisms for this concerted movement and how such movement might regulate Z ring placement are discussed below.

Regulation of Z ring placement by the Min proteins

MinC, MinD and MinE are important regulators of Z-ring placement in *E. coli*. Without MinC, MinD or all three proteins, *E. coli* divides either normally at the midcell position, or abnormally near the cell pole to form a nucleoid-free minicell — hence the name Min proteins. Many genetic and biochemical experiments have provided insights into the way that the Min proteins act to control Z-ring formation: MinC is an inhibitor of FtsZ polymerization, resulting in the inhibition of Z ring assembly in the cell; MinD greatly enhances the inhibitory effects of MinC *in vivo*; and MinE antagonizes the effects of MinC and MinD [1–6].

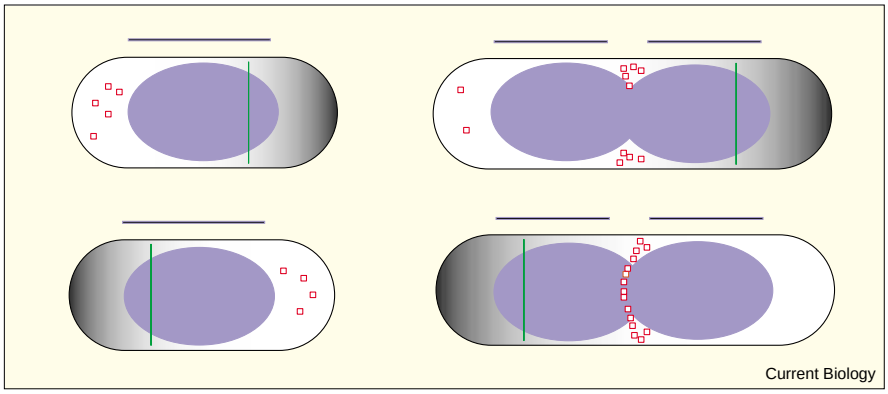
By using fusions to the green fluorescent protein (GFP), it was discovered a few years ago that MinC and MinD rapidly comigrate from one cell pole of *E. coli* to the other [7–9]. The oscillation of the MinC inhibitor is strictly dependent on MinD, an ATPase which recruits MinC to the membrane [3,9,10]. The MinCD complex appears to spend most of its time at one pole or the other in a complex, visible as a single polar zone of localization in each cell. The migration between poles is extremely fast, occurring within a few seconds. The polar zones of MinCD cover a significant fraction of the cell length, appearing as half-cylinders or tubes. The cell poles represent the bottoms of the tubes, and they contain the highest concentration of MinCD; MinCD appears there first and disappears from the poles last. Importantly, this oscillation clearly does not require cell poles or Z rings, because multiple GFP–MinD tubes can oscillate within non-dividing filamentous cells of *E. coli* that lack Z rings. But both the MinCD oscillation and its frequency are dependent on MinE [8].

MinE is a small bifunctional protein. The amino terminus of MinE is required to interact with MinD, while the carboxyl terminus is required for 'topological specificity' — that is, the ability of MinE to antagonize MinCD inhibition of Z rings at the midcell position but not at the poles [1,11]. A functional GFP–MinE fusion protein was initially observed to form a ring at or near the midcell site, independent of FtsZ but dependent on MinD, which persisted at least until the beginning of visible cytokinesis [12]. This finding originally suggested a model in which the MinE ring recognizes a fixed marker at or near the midcell division site and remains there to protect the Z ring from local inhibition by MinCD. To explain why MinE rings are not always at the midcell position, however, another model was proposed in which, instead of being fixed, MinE oscillates along with MinCD, but in a zone closer to the midcell site [13]. This model would also explain the apparent interaction between MinCD and MinE, suggested by yeast two-hybrid data and the ability of MinD to recruit MinE to the membrane [12,14,15].

MinE movement chases MinCD complexes

New work by two different laboratories [16,17] has now demonstrated that MinE–GFP does indeed oscillate along with MinCD. MinE–GFP movement is highly sensitive to the normal balance between MinE and MinD activities, which apparently was not optimal in the initial study. In the new studies, MinE–GFP fluorescence was observed as

Figure 1



Negative regulation of Z-ring positioning by two different systems. The two cells on the left are very early in the cell division cycle, while the two cells on the right are in the process of forming the Z ring. The occlusion by the nucleoid (blue) is represented schematically by blue lines above the cells. The location and the degree of inhibition by oscillating MinCD is represented by the darkness of the grey gradient within the cell. The MinE ring is shown as a green vertical line. FtsZ is shown as red squares, with unassembled FtsZ in the cells on the left, and assembling FtsZ and the Z ring in the cells on the right. Relief of nucleoid occlusion is shown by the gap between the blue lines.

a ring near the midcell site which moves rapidly towards the proximal cell pole, and as a half-cylinder or tube extending from the MinE ring to the proximal pole (the latter localization was also observed in earlier work [12,14]). Although MinC, MinD and MinE have not yet been formally colocalized with separate fluorophores, GFP-tagged MinD and MinE always colocalize to a single polar tube and have similar oscillation times. This strongly suggests that they co-oscillate and implies that the MinE ring marks the rim of a highly dynamic polar tube containing MinC, MinD and MinE. Finally, MinE, like MinCD, can oscillate within multiple nonpolar zones in nondividing filamentous cells, indicating that cell poles and Z rings are not required as physical barriers for the oscillating proteins.

It is probable that the MinD polar tubes consist of a membrane-associated multimeric protein lattice [18]. Without MinE, this MinD lattice is stable and covers the entire cell membrane [14]. This lattice would inhibit cell division everywhere, because MinC colocalizes with MinD and MinC–MinD binding may be especially stable in the absence of MinE [15]. In the presence of MinE, on the other hand, the MinD lattice is unstable but is usually present at one cell pole, and does not dwell at the midcell. This would serve to permanently sequester MinC at alternating cell poles, keeping MinC away from the midcell site most of the time and thus permitting Z-ring assembly there once nucleoid occlusion is relieved (Figure 1).

A model for the oscillation mechanism

How might the oscillation work? MinE appears to disassemble MinD lattices and chase them some distance away to a new site in the cell, where MinD complexes reassemble only to be chased away by MinE again. The existing data are consistent with the following model (Figure 2). As free MinE diffuses from the opposite cell pole, its first encounter with the newly forming MinD lattice is the pole–distal edge of the lattice. Here most MinE molecules rapidly and tightly bind to MinD, forming the MinE ring. Excess MinE molecules diffuse further poleward into the MinD lattice and bind to the higher levels of MinD closer to the pole.

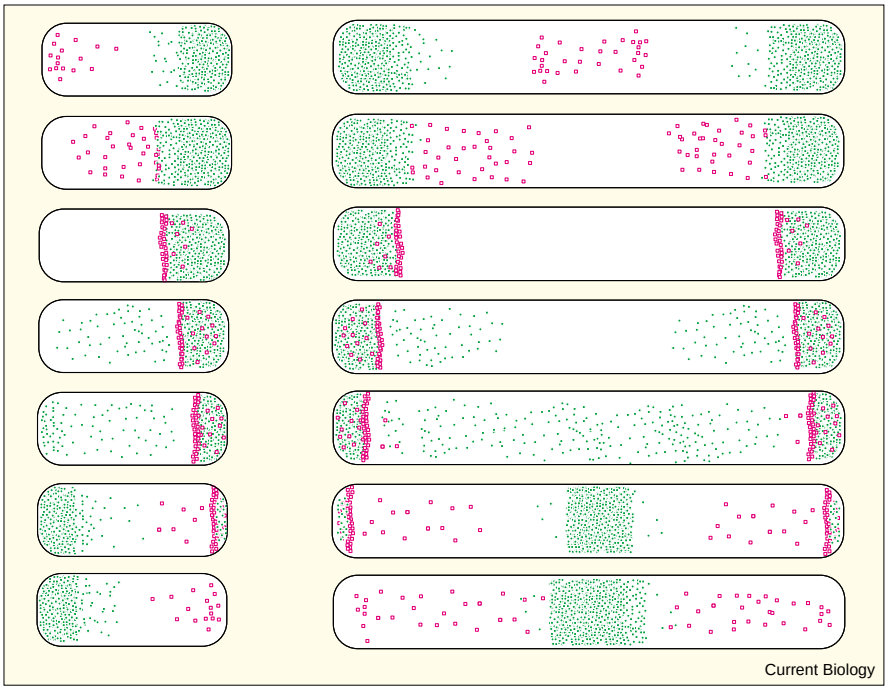
The equilibrium between this diffusion and initial assembly would give rise to the observed MinE ring and the MinE tube. Once formed, the MinE ring rapidly disassembles the MinD lattice, either because MinE concentration is especially high in the MinE ring, or because the ring may be enriched for a dimeric form of MinE [19–21], which might be particularly efficient at MinD disassembly. As the MinE ring disassembles the MinD lattice, it moves, like a snowplow, toward the highest concentration of MinD at the pole, binding and rapidly disassembling the lattice as it goes. The extra-annular MinE may also function in MinD disassembly, but this is not yet clear.

As the old MinD lattice disassembles, free MinD diffuses through the membrane down a MinE concentration gradient until it reaches a sufficiently low concentration of MinE to allow assembly of a new MinD lattice. Although other possible models exist, this one can explain how multiple MinD lattices are able to form away from poles in filamentous cells. In the presence of wild-type MinE, the optimal distance for MinD reassembly from the previous assembly site in filamentous cells is at least 5 μm [16]. This is greater than the length of the average wild-type *E. coli* cell, but it is reasonable to assume that because the pole is an obvious barrier to further MinD diffusion, the lattice is forced to assemble there. In support of this idea, GFP–MinD has been observed to oscillate from pole to pole in dividing cells, which are twice as long as newborn cells [8] (Figure 1).

As the new MinD lattice begins to assemble, the MinE from the previous MinD assembly site disperses, lagging slightly behind MinD. The MinE ring may serve to

Figure 2

A model for Min protein oscillation in *E. coli*. Time courses for a half-cycle of Min oscillation in either a normal cell or a nondividing cell are shown. MinCD complexes, denoted by green dots, first assemble into membrane lattice structures far away from disassembling MinE molecules, which are represented by magenta squares (top cells). As the MinCD lattice assembles, MinE diffuses towards MinCD. Upon reaching the MinCD lattice boundary, MinE assembles into the MinE ring and excess MinE binds to the interior of the MinCD lattice. MinE immediately begins to disassemble the MinCD lattice, causing MinCD to diffuse away from the pole. As the MinE ring plows poleward through the MinCD lattice, MinE begins to diffuse, lagging behind MinCD by a few seconds. MinCD then forms a new lattice away from MinE, and the process repeats.



sequester MinE transiently until MinD has had a chance to start reassembling, thus buying a second or two to allow MinD to escape. The shorter oscillation periods observed with higher MinE:MinD ratios [16] may be indicative of a decreased ability of the MinE ring to block extra MinE molecules from chasing MinD too rapidly. Once the MinE ring disassembles, MinE diffuses, perhaps through the cytoplasm, until it reaches the edge of the new MinD tube. The high affinity between MinD and MinE induces rapid assembly of the MinE ring, and the process repeats.

The recent discovery that all three Min proteins oscillate has shed new light on the general mechanism of the Min system in regulating Z-ring placement. Perhaps the most important new insight is that MinE directly regulates MinCD movement, but only indirectly regulates Z-ring placement via the action of the MinC inhibitor. This further supports the idea that Z rings can assemble anywhere in the cell, except where they are negatively regulated by the independent nucleoid and Min systems [22,23].

Many questions remain, however. How is the oscillation frequency affected by MinD:MinE ratios, the ATPase activity of MinD, or mutants of MinE or MinD? The oscillation becomes much slower at higher MinD:MinE ratios or when carboxy-terminally truncated MinE is used [14]. Does the position of the new MinD assembly site depend on MinE concentration or some other factors? Does the oscillation still occur in branched or spherical *E. coli* cells, which have fewer topological constraints on movement? Can the oscillation be obviated by tethering MinC to the cell pole, in a similar manner to the fixed MinCD polar structures of *Bacillus subtilis*, which lacks MinE? Do other species with MinCDE homologs, such as spherical *Neisseria* species which divide in alternating perpendicular planes [24], also have oscillating complexes, and if so does the oscillation correspondingly switch directions? Finally, a greater understanding of the mechanism of Min oscillation will prove to be useful in understanding other oscillatory protein systems in bacteria that are now being discovered [25–27].

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